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Physicochemical Properties and Storage Stability of Restructured Products Prepared from Mantis Shrimp at Refrigerated Temperature (4±1°C)

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ABSTRACT

The experiment was conducted from January to August, 2023 at Central Institute of Fisheries Education, Mumbai, Maharashtra, India, to evaluate nutritionally rich restructured fish products, such as sausages, using mantis shrimp (*Miyakella nepa*). Ground mantis shrimp meat was incorporated at varying ratios (30%, 50%, and 70%) to replace Pangas fish mince in the restructured fish product. Optimization of the 50% inclusion of shrimp meat in the products was based on physicochemical, rheological, and sensory characteristics, as well as overall acceptability. The optimized restructured fish product in the form of sausages was assessed for quality characteristics over 40 days of refrigerated storage, with a control product containing 100% Pangas mince. Physicochemical parameters did not show significant differences ($p>0.05$) during refrigerated storage. A reduction in gel strength, coupled with increased lipid oxidation, was observed in the products prepared with mantis shrimp meat throughout the storage period. The whiteness of the products decreased slightly, likely due to the spice mix and protein interactions. SDS-PAGE analysis showed no significant changes in protein patterns. However, the mantis shrimp-incorporated sample received a lower overall acceptability score due to the development of a pungent odor by the end of the 40-day storage period.Overall, the study highlighted limited potential for using mantis shrimp in the development of restructured fish products, particularly with fish of high fat content.

KEYWORDS: Mantis shrimp, pangas, sausages, quality, shelf-life

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1. INTRODUCTION

In seafood processing, it is of great interest to increase the yield of marketable products, including developing methods for restructuring low-value cuts and trimmings to improve their appearance, flavour, and texture and enhance the market value (Ramírez et al., 2007). Restructured seafood products are foods created by using minced or chopped seafood muscle, either alone or combined with additional ingredients or additives.(Helena et al., 2016). The basic principle of restructured product is to create a mixture of various meat types or add some plant-based protein and fiber sources (Patel et al., 2023). Restructured seafood products make it possible to provide new different formed and flavoured ready to eat product with high nutritional value to consumers (Tokay et al., 2021). Seafood products are also being elaborated by different restructuring techniques to mimic other more highly-valued products. The main reason for restructuring fish muscle has been due to the limitations on high-value seafood product supplies and, therefore, it becomes necessary to make the best use of existing resources. Many of these resources are under-utilized species that would otherwise have commercial problems because of their size, composition, bony structure, unattractive appearance, or texture (Borderías and Pérez-Mateos, 1996). The utilization of seafood processing waste or discarded species enhances the efficiency of animal protein usage, minimizes environmental problems, and adds a nutritional benefit to diets prepared from such materials (Ozyurt et al., 2019). Usually, the development of the restructured product involves the inclusion of various additives and binding agent (Carpentieri et al., 2022). Additives such as sodium alginate, sodium caseinate, whey protein concentrate, microbial transglutaminase, salt etc, can be used in improving the quality of the restructured product (Gaspar and Goes-Favoni, 2015; Kaewudom et al., 2013; Liu et al., 2019). Among those additives, the use of microbial transglutaminase is found to be most available and efficient alternative for other protein substrates (Gaspar and Goes-Favoni, 2015). Microbial transglutaminase treatment alters the secondary structure, cross-linking, and aggregation of shrimp myofibrillar protein through deamination, enhancing gel properties by catalyzing γ-carboxyl and ε-amino group reactions, increasing disulfide bonds, and improving gel strength (Chen et al., 2024).

The mantis shrimp, often caught as bycatch in shrimp trawling, remains underutilized as food due to its high moisture content, low meat yield after cooking, and difficulty in separating meat from the shell. However, squilla has recently gained importance as an alternative to shrimp seafood in other countries. Against the backdrop of depleting marine resources from Indian waters, developing commercially valuable food products from unconventional resources like squilla requires more attention (Pankyamma et al., 2022). There are the few reports on the development of value-added products from mantis shrimp, such as silage (Bijoy et al., 2016) from squilla and squilla protein powder fortified noodles (Pankyamma et al., 2022) have been reported. Despite having difficulty in consumption of mantis shrimp, they have appreciable level of minerals and their abdomen will be highly desirable for preparing comminuted sausage products due to the better functional properties (Moruf, 2024). Therefore, in this present study, mantis shrimp incorporated restructured product was prepared by optimizing the different combinations of mantis shrimp and pangasius mince and the quality evaluation of optimized mantis shrimp incorporated restructured product was carried out by assessing biochemical, physical and sensory attributes during refrigerated storage at 4±1°C.

2. MATERIALS AND METHODS

The experiment was conducted during January to August, 2023 at Central Institute of Fisheries Education, Mumbai, Maharashtra, India

2.1. Materials

The small eyed squillid mantis shrimp (*Miyakella nepa*) (Latreille, 1828) was procured from the Versova landing center, Mumbai, India between March to May, 2023. The fresh fillets of pangas (*P. hypothalamus*) were purchased from Cambay Tiger, Mumbai. Industrial grade sodium tri–polyphosphate (STPP) and transglutaminase were procured from Sasson Dock Cooperative Fisheries Society Surimi Processing Plant, Uran, Raigarh, Maharashtra, India. Iodized common salt and sugar (Tata Chemicals Ltd., Mumbai, India), sunflower oil (Gold Winner-Kaleesuwari Refinery Pvt Ltd., Chennai), starch and spices including garlic powder, chilli powder, coriander powder, ginger powder, white pepper, etc. were purchased from a local market, Mumbai, India. Analytical–grade chemicals were used during the experiment.

2.2. Methods

2.2.1. Preparation of mantis shrimp meat

The collected mantis shrimp were transported to the laboratory of Post-Harvest Technology, Central Institute of Fisheries Education (CIFE), Mumbai in an icebox packed at 1:1 fish to ice ratio. Mantis shrimp were washed thoroughly with potable tap water on arrival and meat was picked manually with the help of scissors. The picked meat was subjected mechanical deboning machine (Baader 694, Lubeck, Germany) with a drum sieve of 5mm diameter holes to prepare mince. The prepared mince was stored in polyethylene pouches at 4°C until used.

2.2.2. Preparation of pangas mince

The procured fillets of pangas (*P. hypothalamus*) were transported to the laboratory of CIFE, Mumbai in a sterile polythene pack kept inside the icebox at a 1:1 fish to ice ratio. Pangas mince was prepared from eviscerated and cleaned fillets using a mechanical deboning machine (Baader 694, Lubeck, Germany) with a drum sieve of 5mm diameter holes. The temperature was maintained below 10°C throughout the process.

2.2.3. Preparation of mantis shrimp incorporated restructured product

The level of inclusion of fish mince for the preparation of sausage was fixed at 76.5% based on a preliminary study of optimization of the concentration of fish mince. Mantis shrimp mince was incorporated into pangas mince at different levels (30% (T_1), 50% (T_2) and 70% (T_3). The formulations of different treatments are given in Table 1.

The different levels of mixtures of mantis shrimp and pangas mince were then mixed with 0.25% (w/w) sodium tri-polyphosphate (STPP), 1.5% (w/w) iodized common salt, 1% (w/w) sugar and 1% (w/w) transglutaminase. These additives were mixed in silent cutter. After communuting mince mixture for 3 min, 4% (w/w) sunflower oil was added slowly and mixing was continued for another 3 min. Then starch was added at the level of 10% and mixed well. After mixing of oil and starch, a mixture of spices (1.5% (w/w) ginger powder, 1.5% (w/w) garlic powder,1% (w/w) chilli powder, 1% (w/w) coriander powder and 0.75 % (w/w) white pepper powder) were added simultaneously. Each batter was blended for three minutes. The control sausage was prepared without the inclusion of any mantis shrimp mince (C_1) and with mantis shrimp only (C_2). The temperature of the batter was maintained below 15°C throughout the processing. The sausage batters thus obtained were stuffed manually into polyvinylidene casings (diameter: 4cm; length: 17.5cm) using a plastic sausage stuffer and the two ends were tied tightly using strings. The stuffed sausages were cooked at 40°C for 30 min followed by 90°C for 20 min in a thermostatically controlled water bath (Strike 300, Steroglass, Perugia, Italy). The formed sausages were further cooled at once in iced water and stored overnight at 4°C in the refrigerator for further study.

Table 1: Preparation of mantis shrimp incorporated restructured fish product

Ingredients	C_1 (no mantis shrimp meat)	C_2 (100% mantis shrimp meat)	T_1 (30% mantis shrimp meat)	T_2 (50% mantis shrimp meat)	T_3 (70% mantis shrimp meat)
Fish mince	76.5 %	–	49 %	35%	21 %
Mantis shrimp	–	76.5 %	21%	35 %	49 %
Salt	1.5%	1.5%	1.5%	1.5%	1.5%
Sugar	1%	1%	1%	1%	1%
STPP	0.25%	0.25%	0.25%	0.25%	0.25%
TGase	1%	1%	1%	1%	1%
Starch	10%	10%	10%	10%	10%
Spice mix	5.75%	5.75%	5.75%	5.75%	5.75%
Oil	4%	4%	4%	4%	4%

2.3. Analytical methods for quality evaluation

2.3.1. Physicochemical quality

The proximate composition (moisture, crude protein, fat, and ash) of mantis shrimp incorporated restructured product and control sausages was performed according to the standard method (Ruby et al., 2022). The total volatile base nitrogen (TVB-N) was determined based on the method given by (Malle and Tao, 1987). Peroxide value and Free fatty acid were determined following the procedure of Rathod and Pagarkar (2007). Thiobarbituric acid reactive substances (TBARS) were determined as per the method of Tarladgis et al. (1960).

2.3.2. Rheological quality

2.3.2.1. Gel strength

Gel strength was measured by Rheo Tex (Type SD-700, Sun Scientific Co. Ltd, 4-Chome, Kamiyoga, Setagaya-KU, Japan) equipped with round-ended metal probe (5 mm diameter, 60 mm/min) with a load cell of 2 kg. The gel samples were equilibrated at room temperature (25 °C) for 2 h before analysis. The samples were cut into cylinder-shaped samples of 2.5 cm in length for analysis. The load (g as breaking force) and the depth of depression (mm as deformation) when the gel sample lost its strength and ruptured were recorded. All determinations were carried out in triplicates. The gel strength of the sample was calculated as breaking force×deformation and expressed in g.cm.

2.3.2.2. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of sausage gels were analysed by SDS-PAGE (Laemmli, 1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution was added with 3 g of fish sample. The mixture was then homogenized at a speed of 11,000 rpm using a homogenizer for 1 min. The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The samples were centrifuged at 8000×g for 20 min at room temperature (26–28°C) using a centrifuge (Eppendorf 5810R, Germany). Protein concentration in the supernatant was determined as per the Biuret method. Solubilized samples were mixed at a 1:1 (v/v) ratio with the sample buffer (0.5 M Tris-HCl, pH 6.8, containing 4% SDS, 20% glycerol and 10% β-ME) and boiled for 3 min. Samples (5 µg protein) and high molecular weight (10–315 kDa) protein marker (Genetix, Biotech Asia Pvt. Ltd., India) were loaded onto polyacrylamide gels comprising a 10% running gel and a 4% stacking gel and subjected to electrophoresis at a constant current of 15 $mAgel^{-1}$ using a Hoefer unit (Hoefer, Inc., San Francisco, CA, USA). After electrophoresis (Electrophoresis Power supply- EPS601, China), the gel was stained with 0.02% (w/v) Coomassie Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and de-stained with 50% (v/v) methanol and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.3.3. Whiteness

The whiteness of mantis shrimp incorporated restructured product was measured using a Nippon Denshoku Colourimeter (ZE 6000). Whiteness was calculated as described by Park (1994) as follows:

Whiteness index=100-($\sqrt{(100-L^*)^2+(a^*)^2+(b^*)^2}$

2.3.4. Microbiological examination

The total plate count of the mantis shrimp incorporated fish sausage was done by spread plate technique (BAM, 2004). 10 g of sausage sample was taken aseptically and transferred into a sterile stomacher bag and it was mixed with 90 ml sterile saline. The mixture was homogenized for 60 seconds in a stomacher. Decimal dilutions were prepared and 100 microliter from each dilution was spread on sterile plate count agar plates. The plates were incubated at 37°C for 24 hours. The plates were enumerated and results were reported as log colony forming unit per gram (log cfu g^{-1}).

2.3.5. Sensory evaluation

Sensory characteristics of sausage samples for each treatment group were evaluated by experts, who were trained and familiar with sausage consumption. Sausages were cut into thin slices and served to panelists for evaluation of their colour, odour, taste, texture, flavour, appearance and overall acceptability based on a 9-point hedonic scale (Mailgaad et al., 1999). Panel members were asked to assign scores based on the following scale, 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely.

2.3.6. Shelf-life study

Analysis of the shelf life of chosen mantis shrimp incorporated restructured product was carried out once in 10 days for 40 days. The optimized (50%) mantis shrimp incorporated restructured product along with control were prepared and stored in refrigerated condition at 4±1°C for shelf-life study. Samples were evaluated for physicochemical, rheological, whiteness, microbiological and sensory parameters for 40 days at 4±1°C.

2.3.6. Statistical analysis

All the experiments were carried out in triplicates and results were expressed as Mean±Standard Deviation (S.D). Analysis of Variance was carried out using a Statistical package for social science software (SPSS VERSION 25.0, Chicago IL, USA).The level of significance was set up at $p \leq 0.05$. Duncan's Multiple Range Test (DMRT) was carried out to find out the statistically significant difference.

3. RESULTS AND DISCUSSION

The present study of the inclusion of different proportions (30, 50 and 70%) of mantis shrimp meat in the development of restructured fish product i.e. sausage was conducted for optimization of the concentration of mantis shrimp meat in the Pangas sausage to make the product acceptable.

3.1. Optimization of concentration of mantis shrimp meat in restructured fish products

3.1.1. Proximate composition of restructured fish products

The proximate composition of the optimized restructured product from mantis shrimp is depicted in Table 2.

The highest moisture content in C_2 (66.09%) was due to the high moisture content of mantis shrimp meat (81.47%). As the percentage of inclusion of mantis shrimp meat increased, the moisture content of the restructured products also increased. The protein content in C_1 (15.57%) was higher than other treatments. This was due to the higher protein content of pangas meat (17–19%) (Sokamte et al., 2020) than mantis shrimp meat (15.06%). The fat content was higher in C_1 (6.26%). The incorporation of higher levels of mantis shrimp in pangas mince decreased the fat content of the resultant products. This may probably be due to the presence of higher amount of fat in pangas meat (1%) (Sokamte et al., 2020) than the fat content of mantis shrimp (0.66%). In the case of ash content, higher ash content was

Table 2: Proximate composition of restructured fish product

Treatments	C_1	C_2	T_1	T_2	T_3
Moisture (%)	59.62±0.19[a]	66.09±0.02[e]	61.22±0.10[b]	61.93±0.14[c]	62.91±0.04[d]
Protein (%)	15.57±0.41[c]	10.70±0.45[a]	14.50±0.32[b]	14.09±0.57[b]	14.55±0.27[b]
Fat (%)	6.26±0.05[e]	3.89±0.12[a]	5.18±0.10[d]	4.90±0.14[c]	4.38±0.13[b]
Ash (%)	2.60±0.02[a]	3.03±0.03[c]	2.87±0.07[b]	2.92±0.06[b]	3.00±0.06[c]

Values (n=3) are presented as the mean±SD. The mean value in the same row with different superscripts is significantly different between the treatments (p<0.05)

found in C_2 (3.03%) and T_3 (3.00%), which could be due to higher ash content in mantis shrimp meat (2.26%) than that of pangas meat (1.24%) (Sokamte et al., 2020).

3.1.2. Gel strength of restructured fish products

The gel strength of the optimized restructured product is shown in Table 3.

Table 3: Rheological characteristics and whiteness of restructured fish product

Treatments	Gel strength (g.cm)	Whiteness
C_1	615.57±5.16[e]	9.22±0.15[b]
C_2	74.22±2.44[a]	8.33±0.15[a]
T_1	328.81±6.25[c]	8.59±0.14[a]
T_2	367.18±2.87[d]	8.32±0.18[a]
T_3	238.73±1.93[b]	8.24±0.03[a]

Values (n=3) are presented as the mean±SD. The mean value in the same row with different superscripts is significantly different between the treatments (p<0.05)

The experimental results showed a significant difference (p<0.05) in the gel strength of Pangas sausage after the addition of mantis shrimp meat. Mantis shrimp meat had the lowest gel strength (74.22 g·cm) in the present study. The low gel-forming ability of the shrimp meat was likely due to its high cysteine protease activity (Takahashi et al., 2014), which affected the overall gel strength of the pangas restructured product. In accordance with Mehta and Nayak (2017), the gel strength of fresh white-leg prawns was found to be 80.60 g·cm. Similarly, the gel obtained from pink prawns (*Pandalus* sp.) was also believed to possess lower gel strength (Takahashi et al., 2014).

3.1.3. Protein patterns of formulated sausages

The SDS–PAGE protein patterns of mantis shrimp incorporated restructured product are shown in Figure 1. In C_1, T_1, and T_2, the intensity of the MHC band (245kDa) was higher than other treatments. The MHC content of all samples gradually reduced with the increase in concentration of mantis shrimp inclusion in products. This suggested that the increased addition of mantis shrimp meat reduces the formation of intermolecular covalent bond cross-linking

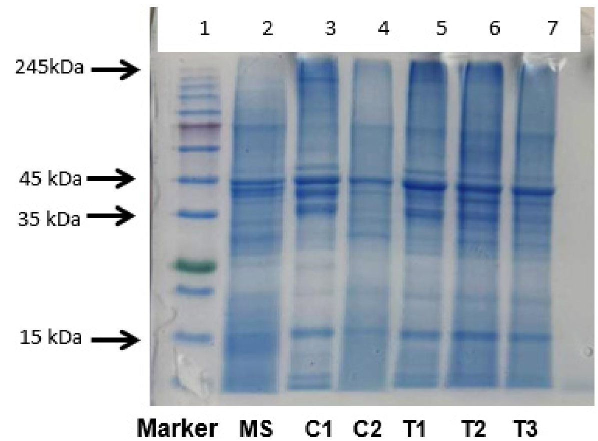


Figure 1. SDS–PAGE pattern of proteins of restructured fish products. lane 1: marker, lane 2: MS mantis shrimp meat, lane 3: C_1 100% Pangas mince, lane 4: C_2 100% Mantis shrimp meat, lane 5: T_1 30% Mantis shrimp meat, lane 6: T_2 50% Mantis shrimp meat, lane 7: T_3 70% Mantis shrimp meat)

of MHC. Moreover, MHC strips were almost invisible in C_2 and T_3. Among the treatments, C_1 showed a prominent band of 45 kDa when compared to C_2. As the concentration of mantis shrimp meat incorporation increased, the band intensity of 45 kDa was reduced. In C_1, T_1, and T_2 sausages, a band of 35 kDa showed some intensity when compared to others.

3.1.4. Whiteness of restructured fish products

The intention of the consumer to purchase a product or not depended on the general perception of the colour characteristics of the product. The whiteness of the optimized treatments is depicted in Table 3. In the present study, the whiteness of the product was found to have reduced due to the addition of a coloured spice mixture. These results were in agreement with Majumdar et al. (2015), who reported that the addition of garlic extract was responsible for the darkening of *Pangasius* surimi sausage. Additionally, there was a possibility of interaction between spice mix compounds and muscle pigments, leading to a reduction in the whiteness of the sausage. The difference in colour between the control and treatments was possibly caused by differences in the pigment content in the muscles of Pangas and mantis shrimp. The reduced whiteness of the treated product was likely due to the presence of the protein-astaxanthin complex in mantis shrimp. This complex usually dissociated during cooking, allowing free astaxanthin to reveal its colour, ranging between yellow, orange, and red (Weesie et al., 1995).

3.1.5. Sensory analysis of restructured fish products

The palatability of meat products is a complex characteristic that is influenced by various factors, including odour and flavour, color, appearance, tenderness, and juiciness. Among the aforementioned factors, consumers tend to assign greater importance to flavour and texture. The sensory analysis of formulated sausages is shown in Table 4.

The results of the present study indicated that the addition of mantis shrimp in restructured products influenced flavour and overall acceptability of the product. Other parameters like appearance, texture, odour, taste and colour did not show any significant difference (p>0.05). The score for flavour was higher in C_1. The inclusion of mantis shrimp meat in the products affected flavour characteristics in the present study. The results showed that the inclusion of 50% mantis shrimp in the development of restructured products was highly accepted compared to other treatment and control groups.

Based on the results of sensory evaluation, the Pangas sausages containing 50% mantis shrimp meat showed a higher overall acceptability score from the consumer point of view. Therefore, Pangas sausages containing 50% mantis shrimp meat (S_1) was considered as optimized concentration for the development of restructured fish product and considered for further study. The physicochemical, rheological and microbial quality of S1 was compared with control sausages (S0) containing 100% Pangas mince for 40 days in refrigerated storage.

Table 4: Sensory analysis of the restructured fish product

Treatments	Appearance	Texture	Colour	odour	Taste	Flavour	Overall acceptability
C_1	8.00±0.80^{a}	7.12±1.35^{a}	8.12±0.35^{a}	8.25±0.65^{a}	8.25±0.37^{a}	8.21±0.64^{b}	7.92±0.17^{bc}
C_2	8.00±0.75^{a}	5.93±1.01^{a}	7.87±0.64^{a}	8.25±0.75^{a}	8.12±0.69^{a}	7.71±0.92^{ab}	7.50±0.59^{a}
T_1	8.12±0.69^{a}	6.75±1.06^{a}	8.00±0.46^{a}	8.18±0.65^{a}	8.06±0.17^{a}	7.85±0.44^{ab}	7.92±0.17^{bc}
T_2	8.12±0.69^{a}	6.56±1.17^{a}	8.00±0.53^{a}	8.31±0.70^{a}	8.25±0.46^{a}	7.50±0.53^{a}	8.14±0.34^{c}
T_3	8.15±0.53^{a}	6.43±0.97^{a}	7.81±0.84^{a}	8.12±0.83^{a}	8.12±0.64^{a}	7.50±0.46^{a}	7.71±0.36^{ab}

Values (n=10) are presented as the mean±SD. The mean value in the same row with different superscripts is significantly different between the treatments (p<0.05)

3.2. Shelf life study of final restructured product under refrigerated storage (4±1°C)

3.2.1. Effect of mantis shrimp inclusion on proximate composition

The changes in the proximate composition of the restructured products during refrigerated storage are shown in Table 5.

Table 5: Proximate composition of the final restructured product during refrigerated storage (4°C)

Storage period (days)	Treatments	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
0th day	S_0	58.80±0.36^{a}	15.65±0.36^{c}	6.15±0.55^{a}	2.98±0.23^{a}
	S_1	61.45±0.45^{c}	13.18±0.27^{b}	4.68±0.22^{a}	2.88±0.05^{a}
10th day	S_0	58.42±0.17^{a}	15.24±0.00^{b}	6.10±0.45^{a}	2.91±0.36^{a}
	S_1	60.91±0.21^{b}	11.85±0.08^{a}	4.79±0.52^{a}	2.85±0.13^{a}
20th day	S_0	56.27±0.48^{a}	14.94±0.04^{b}	6.01±0.37^{a}	2.80±0.10^{a}
	S_1	60.21±0.03^{a}	11.92±0.84^{a}	4.76±0.17^{a}	2.87±0.18^{a}
30th day	S_0	56.10±0.91^{a}	14.87±0.09^{b}	6.03±0.09^{a}	2.79±0.17^{a}
	S_1	60.17±0.14^{a}	11.47±0.23^{a}	4.65±0.35^{a}	2.85±0.09^{a}
40th day	S_0	56.00±0.19^{a}	14.40±0.24^{a}	5.89±0.28^{a}	2.70±0.18^{a}
	S_1	60.10±0.14^{a}	11.42±0.16^{a}	4.44±0.20^{a}	2.83±0.18^{a}

Values (n=3) are presented as the mean±SD. The mean value in the same row with different superscripts is significantly different between the treatments (p<0.05)

Significant differences (p>0.05) were not observed in the moisture, fat, and ash content of control sausages and in the fat and ash content of mantis shrimp incorporated restructured product during refrigerated storage of 40 days. However, there was a decreasing trend in the moisture content of treatment sausage samples. Similarly, a significant reduction in moisture content of the samples stored at 4°C was reported for dry sausage prepared from silver carp during refrigerated storage (Oksuz et al., 2008). Also, the reduction of moisture content was noticed in sausage prepared from lantern fish (*Benthosema pterotum*) using fish protein isolate during storage (Moosavi-Nasab et al., 2018). The steady moisture content was noticed by Rahmanifarah et al. (2015) while studying the quality of fish sausage from silver carp during refrigerated storage. In the present study, the protein content of both control and treatment sausages was reduced over the storage period. The decrease in protein content in the rohu sausage due to the addition of corn flour, hydrogenated oil and other ingredients was reported (Sini et al., 2008).

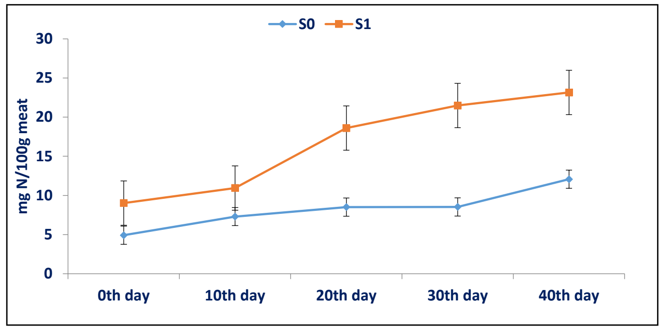
3.2.2. Effect mantis shrimp inclusion on the chemical quality

Total volatile base nitrogen (TVB-N) is often used as an indicator of the degree of spoilage in seafood quality (Jeyakumari et al., 2016). The effect of inclusion of mantis shrimp on TVB-N of restructured product during storage is shown in Figure 2a.

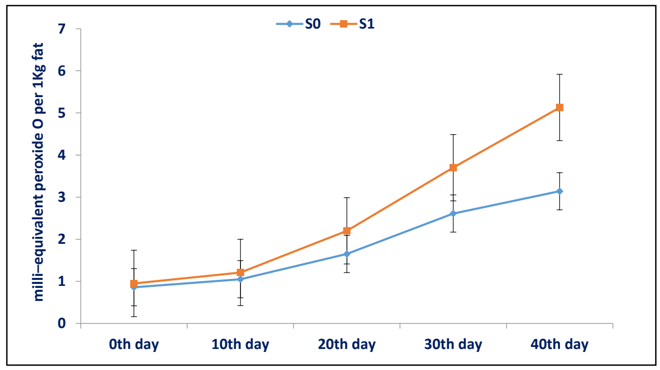
In the present study, initially, TVB-N showed a slightly increasing trend, but an abrupt rise in TVB-N after 30 days of storage of S1, indicating microbial oxidation of protein at later stages. The increase in TVB-N was due to the volatile bases resulting from the degradation of protein compounds and non-protein nitrogenous compounds mainly due to microbial activity (Connell, 1975).

Peroxides are the primary lipid oxidation products and play a central role in the auto-oxidation of lipids and decompose them into carbonyls and other compounds (Jeyakumari et al., 2016). The changes in the peroxide value of restructured products during refrigerated storage are shown in Figure 2b. The results of the present study showed an increasing trend in peroxide values (PV) during refrigerated storage. The high peroxide values were found in the treatment group compared to the control group. However, the PV values in all sausages were much lower than the acceptance limit of 20 meq O_2 kg^{-1} oil throughout the period of storage study.

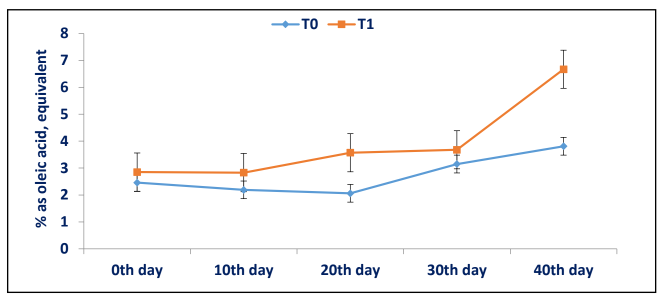
During refrigerated storage, lipids have a tendency to undergo hydrolysis, leading to the production of free fatty acids (FFA). The presence of free fatty acids (FFA) was responsible for the alterations in texture, increased lipid oxidation, and the emergence of undesirable flavors in muscle-based food products (Sequeira-Munoz et al., 2006). The effects on the free fatty acid content of restructured



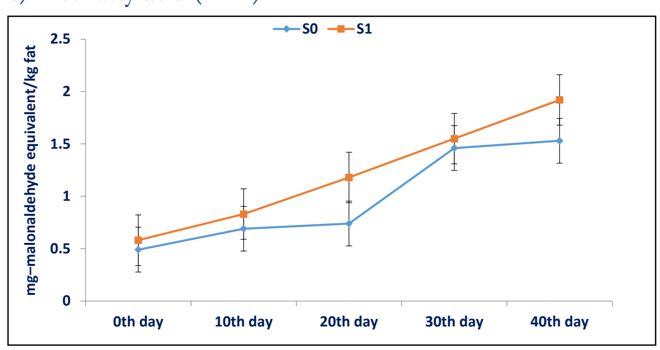
a) Total volatile basic nitrogen (TVB–N)



b) Peroxide value (PV)



c) Free fatty acid (FFA)



d) Thiobarbituric acid reactive substances (TBARS)

Figure 2: Changes in chemical quality of final restructured product during refrigerated storage (4°C)

products during storage are shown in Figure 2c. In the present study, lipid hydrolysis occurred to a greater extent at the end of the 40th day. However, the change in FFA values in the control was significantly lower than treatment sample throughout the storage period. Since the release of FFA content increased at the end of storage days, as found in this study, it is reported that there is a relationship between FFA release and loss of freshness of fish products (Ozogul et al., 2005; Rodriguez et al., 2006). During chilling storage, FFA formation has been reported to be produced during the first stage as a result of endogenous enzymes, namely, lipases and phospholipase activity (Whittle et al., 1990). Later on, microbial activities play an important role in FFA formation as a result of bacterial enzyme activity. Therefore, FFA values were in correlation with microbiological counts in the present study.

The thiobarbituric acid (TBA) test has been widely used to measure the degradation of products of lipid hydroperoxides formed during lipid oxidation. The TBA test mainly determines the malondialdehyde (MDA), which is derived from the oxidation decomposition of unsaturated fatty acids (Fernández et al., 1997). The effects on TBARS content of restructured products during storage are shown in Figure 2d. A significant increase in TBARS value in control and treated samples was observed during the entire storage period. However, the change in TBARS value in the control was significantly lower than the treatment sample throughout the storage period. The increase in TBARS value may be due to the decomposition of unstable hydroperoxides, which results in the production of secondary products of oxidation (Haghshenas et al., 2015).

3.2.3. Effect of mantis shrimp inclusion on gel strength

The changes in the gel strength of restructured products during refrigerated storage are presented in Table 6. As observed in the optimization of the concentration of mantis shrimp inclusion, the addition of mantis shrimp meat significantly reduced the gel strength of the treatment sausages compared to the control sausage. From the results of the storage study, it can be observed that the gel strength did not show any significant difference (p>0.05) at refrigerated temperature. Usually, the folding strength can directly reflect the gel strength of fish sausage and allow researchers to quickly understand its texture characteristics (Surasani et al., 2022).

Table 6: Rheological characteristics and whiteness in final restructured product during refrigerated storage (4°C)

Storage period (days)	Treatments	Gel strength	Whiteness
0th day	S_0	778.99±60.04^a	9.38±0.15^b
	S_1	498.56±19.58^a	8.64±0.02^b
10th day	S_0	NP	NP
	S_1	NP	NP
20th day	S_0	760.22±66.24^a	9.22±0.49^{ab}
	S_1	496.92±15.60^a	8.55±0.08^b
30th day	S_0	NP	NP
	S_1	NP	NP
40th day	S_0	725.49±56.45^a	8.63±0.17^a
	S_1	481.02±46.40^a	8.25±0.06^a

Values (n=3) are presented as the mean ± SD. The mean value in the same row with different superscripts is significantly different between the treatments (p<0.05); NP: Not performed

3.2.4. Effect of mantis shrimp inclusion on SDS–PAGE pattern of proteins

The effect of refrigerated storage on the SDS–PAGE pattern of proteins of restructured product is shown in Figure 3.

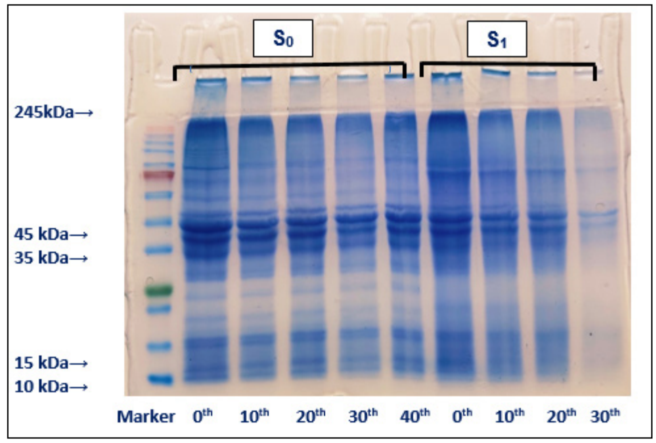


Figure 3: SDS-PAGE pattern of proteins of final restructured product during refrigerated storage (4°C); S0: Control (100% Pangas mince), S_1: Treatment (50% Mantis shrimp)

The present study demonstrated that the mantis shrimp incorporated sausage exhibited increased proteolytic degradation as the storage period increased. Changes in fish meat during post-mortem storage included alteration of myosin-actin interactions, loss of z-line structure and also lead to degradation of the protein (Briskey and Fukazawa, 1971).

3.2.5. Effect of mantis shrimp inclusion on whiteness

In the present study, the whiteness of the restructured product during refrigerated storage is presented in Table 6.

The results regarding changes in colour attributes of sausages under refrigerated storage displayed a little but significant decrease in whiteness. Such reduction in whiteness may be related to the formation of compounds formed during lipid oxidation. Similarly, a reduction in whiteness during storage was observed in *Pangasius* surimi sausage with garlic aqueous extract (Majumdar et al., 2015).

3.2.6. Effect of mantis shrimp inclusion on microbiological quality

The initial TPC of control (S_0) and treatment (S_1) are

2.17 and 2.54 CFU g^{-1} respectively. A steady increase was observed in the TPC of samples during the storage period of 40 days. At the end of the 40th day of storage, TPC was increased to 3.89 and 4.98 CFU g^{-1} for control (S_0) and treatment (S_1) respectively. The rate of increase in CFU g^{-1} was more in treatment (S_1) than in control (S_0). This may be due to the presence of a large number of free amino acids and nitrogenous compounds in crustacean meat, which can serve as nutrients for microorganisms (Sae-Leaw and Benjakul, 2019).

3.2.7. Effect of mantis shrimp inclusion on sensory quality

Changes in the sensory attribute of the restructured products during refrigerated storage are represented in Figure 4.

From the figure, it was observed that on the 0th day, all sensory attributes received excellent scores, but as the storage period progressed, the scores for all the attributes decreased.

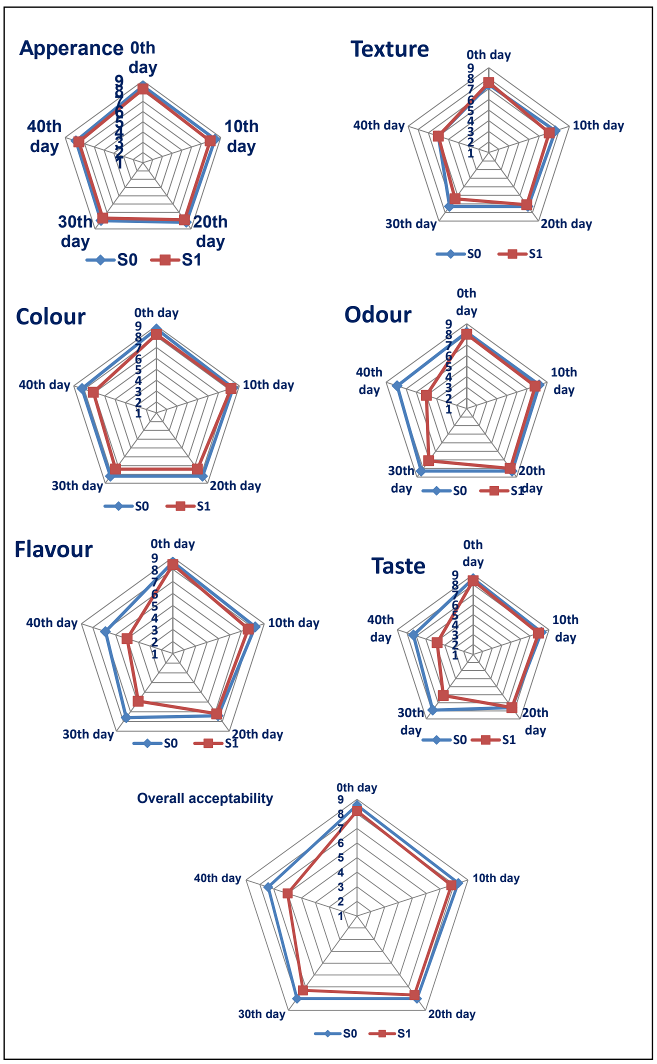


Figure 4: Radar diagram displaying changes in sensory score of the final restructured products during refrigerated storage (4°C)

By the 40th day of refrigerated storage, the control sausage had a higher overall acceptability score (7.40) compared to the treatment sausage (6.00). This was likely due to the presence of a pungent odour in the treatment sausage, which resulted from increased microbial growth.

4. CONCLUSION

The addition of mantis shrimp significantly improved the sausage quality in terms of consumer acceptability. Lipid deterioration and a loss in whiteness were observed during the refrigerated storage of sausages. The gel strength and folding score did not show any significant differences throughout the storage period. Finally, it was inferred that incorporating mantis shrimp at a level of 50% in Pangas mince yielded a good and healthy ready-to-eat fish-based product like sausage

5. ACKNOWLEDGEMENT

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